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(54) Title: METHOD OF DETECTION OF BACTERIA

(57) Abstract

The invention relates to a method for detecting particular antigens consisting of microorganisms (e.g. yeast) or more preferably bacteria such as, for example, those of the genus Salmonella, Listeria, E. Coli or Staphylococcus present in a mixed culture, in a pure culture or in a contaminated food sample. This invention utilises immunological complex formation as a measure of the amount of antigens present by means of the use of specific antibodies including monoclonal antibodies belonging to the class of proteins called immunoglobulins (molecules Ig) labelled with chemiluminescent molecules consisting of acridinium derivatives. This invention further concerns kits for performing tests according to said method.

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METHOD OF DETECTION OF BACTERIA

BACKGROUND OF THE INVENTION

I. Field of the invention :

The invention relates to a new method for detecting particular antigens consisting of microorganisms (e.g. yeast) or more preferably bacteria such as, for example, those of the genus Salmonella, Listeria, E. Coli or Staphylococcus present in a mixed culture, in a pure culture or in a contaminated food sample. This invention utilises immunological complex formation as a measure of the amount of antigens present by means of the use of specific antibodies labelled with chemiluminescent molecules. This invention further concerns kits for performing tests according to said method.

II. Discussion of the prior art :

Bacteria such as Salmonella species exist in a range of environments but impose the greatest danger to health when it is found in food and feedstuffs. Poultry, meat and eggs are common sources of Salmonella. When the bacteria is consumed, it is able to establish itself in the gut and multiply, resulting in the appearance, several days after the initial ingestion, of clinical symptoms including vomiting, diarrhoea and nausea, and in severe cases said symptoms may result in death.

It is therefore highly desirable to provide test methods by means of which such dangerous antigens may be detected. Among the various methods disclosed in the prior art, solid-phase immunoassays, based in particular on enzymes as labels of the antigen-antibody immune complex (ELISA technique), have found wide application in diagnostic microbiology due to their high specificity (see in particular the reference WO-A-89/01162). WO-A-87/03690 discloses an immunoassay method for the detection and quantification of certain analytes, such as a virus. WO-A-92/14156 discloses a method for detecting of mycobacteria in biological fluids. EP-A-0429794 discloses an assay method for detecting the presence of listeria strains, as does EP-A-0498920. The use of a chemilumnometric sandwich immunoassay for detecting chlamydia trachomatis antigen is described in "Sexually Transmitted Diseases" 1992, Vol 19(3), pages 161 - 164.

However the ELISA technique is characterized by a level of sensitivity for the detection of the antigens which remains to be improved.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an immunological method for detecting particular antigens consisting of microorganisms (e.g. yeast) or more preferably bacteria such as, for example, those of the genus Salmonella, Listeria, E. Coli or Staphylococcus, which allows improved levels of sensitivity which may be in the region of about from 100 to 1000 times greater than the ELISA technique.

With such an increase, it becomes possible to detect the presence or absence of bacteria after much shorter time periods for the preparation of samples prior to testing , and this constitutes another object of the present invention ; said prior preparation is made in order to allow the number of bacteria to increase in order to reach a detectable level, and the requisite time for performing said enrichment of bacteria may be lowered to a value of approximately 24 hours compared to the common 44 hours for ELISA.

Another object of the present invention is to provide a method having the above-mentioned advantages, which may be carried out without requiring a particular high skill level on the part of the operator.

DETAILED DESCRIPTION OF THE INVENTION

A - Specifically, in one aspect the present invention provides an immunological method for detecting a particular antigen consisting of bacteria present in a mixed culture, in a pure culture or in a contaminated food sample, which comprises the following steps :

a) bringing the sample to be tested into contact with an appropriate solid phase, during a first incubation time sufficient to allow the antigen to be immobilized upon or inside the structure of said solid phase,

b) subjecting the solid phase incubated in the previously described manner to a first wash step to remove the unbound antigen matter,

c) bringing the washed solid phase into contact with a specific antibody, which is labelled by coupling to a chemiluminescent agent comprising an acridinium derivative, the coupling being either in situ or in an extemporaneous

manner, said contact being performed during a second incubation time sufficient to allow the labelled antibody to bind to the antigen.

d) subjecting the solid phase (associated in the previously described manner to the complex consisting essentially of the labelled antibody bound to the antigen) to a second wash step to remove any unbound labelled antibody, and

e) placing the solid phase obtained as previously described under point d) above under conditions whereby light may be produced, and the luminescence activity produced may be quantitatively measured with the help of an appropriate device.

More specifically, the present invention provides a method for detecting particular antigens consisting of bacteria of the genus Salmonella, Listeria, E. Coli or Staphylococcus. Said method is particularly suitable for detecting Salmonella.

I . In a first embodiment, the present invention provides a method which is performed by using, under point a), an "appropriate solid phase" which includes a solid substrate in the form of either a bead, a tube or a microtiter plate well, which may be made of material such as, for example, polystyrene, polyvinyl chloride, nylon, titanous hydroxide, agarose beads or cellulose derivatives.

According to a preferred aspect of this first embodiment of the present invention, the appropriate solid phase comprises a microtiter plate well. In a more preferred aspect, the microtiter plate well is made of polystyrene.

The exposure of the sample (which comprises the antigens to detect) to the solid phase or the first incubation time may vary between about 15 minutes to about 3 hours, and preferably between 20 minutes to about 2 hours 30 minutes, at a temperature in the range of about 20°C to about 40°C and generally in the presence of a buffer which is preferably a saline buffer giving a slightly alkaline pH.

Generally, the wash steps described under points b) and d) are each performed by using a buffer and preferably a saline buffer also giving a slightly alkaline pH.

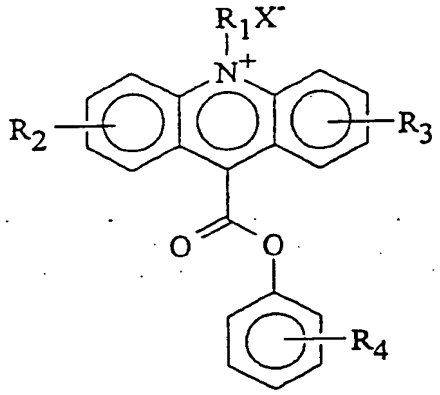
The antibody which is used under point c) is an antibody which will bind to native or denatured bacteria with sufficient specificity to provide a useful assay.

The antibody which is preferably used includes monoclonal antibody belonging to the class of proteins called immunoglobulins (molecules Ig) derived from animal sources, such as primarily vertebrates and preferably mammals. The antibody which is more preferably used consists of a monoclonal antibody belonging to the class of proteins called immunoglobulins gamma (molecules IgG)

derived from rabbits. The antibody is made by conventional techniques known per se.

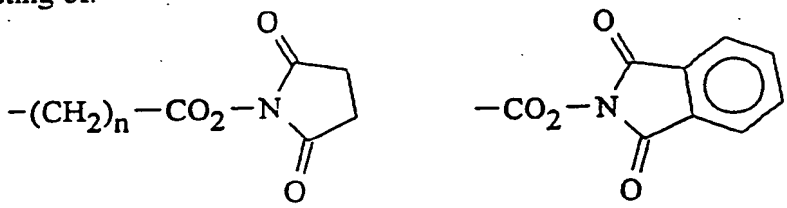
The chosen antibody is labelled with a chemiluminescent agent consisting of acridinium derivatives such as, for example, those described in the book "Comprehensive Analytical Chemistry", edited by G. Svehla, volume XXIX headed "Chemiluminescence Immunoassay" by I. Weeks, published by Elsevier in 1992, pages 225 to 255. Chemiluminescent labels are also described in EP-A-0263657.

Preferably, the labelling step is performed by using an acridinium derivative comprising a substituted phenyl ester of an acridinium carboxylic acid having the general formula (I) :



(I)

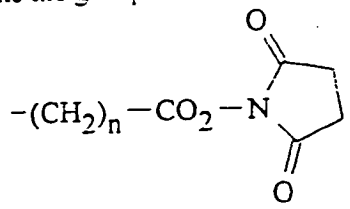
in which : R_1 represents hydrogen, C_1 - C_{10} optionally substituted alkyl, alkenyl or aryl group ; X represents an acceptable salt-forming anion including, for example, a chloride, bromide, fluoride, sulphite, sulphate, bisulphite, phosphate, orthophosphate, carbonate, bicarbonate, benzoate, sulphonate, methylsulphonate, methylfluorosulphonate ; R_2 and R_3 are hydrogen, amino, substituted amino, carboxyl, alkoxy, nitro or halide substituents ; R_4 is selected from the group consisting of:



-NCS, $-C(=NH_2^+ X^-)-OR_5$, halide and azide, wherein n represents zero or an integer from 1 to 6 and R_5 represents groups such as R_1.

More preferably, the labelling step is performed by using an acridinium derivative consisting of a substituted phenyl ester of 10-methylacridinium-9-

carboxylic acid corresponding to formula (I) wherein R_1 is CH_3, R_2 and R_3 are hydrogen, R_4 represents the group :



wherein n represents zero or an integer from 1 to 4, and X represents one of the salt-forming anions such as those previously defined. The acridinium esters of formula (I) which are used to carry out the method of the present invention are known compounds, the preparation of which is described in the reference EP-A-0082 636.

A general scheme for labelling the antiboby with the acridinium derivative involves the following methodology :

- solubilizing the antibody in a saline buffer at a slightly alkaline pH ;
- adding said antibody solution to the label (3 to 10 moles per mole of antigen protein) optionally in solution in an organic solvent, and mixing the ingredients thoroughly;
- leaving the mixture to stand in the dark at a temperature of about 20-30°C for a period of about 5 minutes to about 30 minutes ;
- adding thereafter a quenching buffered compound, and leaving the resulting mixture to stand for a further period of about 5 minutes to about 30 minutes.
- optionally followed by purifying the labelled antibody to remove the excess of labelling compound by gel permeation chromatography.

The exposure of the labelled antibody to the antigen immobilized upon or inside the structure of the solid phase or the second incubation time may vary between about 15 minutes to about 3 hours, and preferably between 20 minutes to about 2 hours 30 minutes, at a temperature in the range of about 20°C to about 40°C and generally in the presence of a buffer which is preferably a saline buffer giving a slightly alkaline pH.

Concerning the procedure to carry out the second incubation step described hereinbefore under point c), it is preferably performed by preparing first in an extemporaneous manner the labelled antibody, and then by adding said labelled antibody to the solid phase comprising the antigen to detect.

In the final step of the method of the invention as previously described hereinbefore under point e), the acridinium derivative moiety of the complex (consisting essentially of the labelled antibody bound to the antigen to detect)

associated with the solid phase, undergoes a light emitting reaction by subjecting said complex-associated solid phase to the action of an activating solution which may be a dilute aqueous solution of an alkaline hydroxide and of a mineral or organic peroxide such as, for example, hydrogen peroxide, and the amount of the acridinium derivative moiety can be quantified by measurement of the intensity of the emitted light by means of a photon counter.

The appropriate device which may be used in the invention to measure the photon emission is that called luminometer ; the level of acridinium moiety which is proportional to the level of antigen can be determined by the relative light units (RLU) registered on the luminometer. A highly contaminated sample will give a high RLU count, whereas a negative sample will give a very low RLU count.

II . In an alternative embodiment, the invention provides a method according to which the detection of the particular antigens may be performed by carrying out an immunoassay called sandwich-immunoassay.

Such a method comprises the various steps which have been previously described hereinbefore under points a) to e), with the following difference : in the first step under point a), the solid phase includes now a solid substrate in the form of either a tube or a microtiter plate well, which is pre-coated, in a manner known per se, with a first unlabelled antibody. Said first antibody may be identical to or different from the second antibody which is labelled with the acridinium derivative as it is stated under point c).

III . In another embodiment, the present invention provides a method which is performed by using, under point a), an "appropriate solid phase" which further comprises a solid substrate in the form of a membrane which may be made of material such as, for example, cellulose acetate, nitro cellulose, polyvinylchloride, teflon, polysulfone, polyester, polycarbonate or polyamide.

According to a preferred aspect of this another embodiment of the present invention, the appropriate solid phase comprises a membrane made of for example cellulose acetate, nitrocellulose or polyamide.

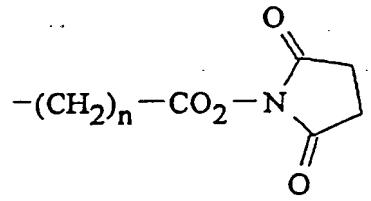
Such a method also includes the various steps which have been previously described hereinbefore under points a) to e), with the following differences :

* the first and second incubation times as previously described under points a) and c) may vary now between about 30 secondes to about 3 hours, and preferably between 1 minute to about 2 hours 30 minutes ;

* the wash steps as previously described under points b) and d) are now performed in conditions whereby in each case the washing agent is pushed through the membrane by means of, for example, vacuum or reduced pressure.

B - Specifically, in another aspect the present invention provides a kit for detecting particular antigens consisting of microorganisms (e.g. yeast) or most preferably bacteria present in a mixed culture, in a pure culture or in a contaminated food sample, which comprises :

- a solid phase, preferably in the form of either a microtiter plate well or a membrane ;
- an antibody (preferably a monoclonal antibody) of the IgG type labelled by coupling with an acridinium derivative (preferably comprising a substituted phenyl ester of 10-methylacridinium-9-carboxylic acid corresponding to formula (I) wherein R_1 is CH_3, R_2 and R_3 are hydrogen, R_4 represents the group :



wherein n represents zero or an integer from 1 to 4, and X represents one of the salt-forming anions such as those previously defined);

- optionally a dilute aqueous solution of an alkaline hydroxide and of hydrogen peroxide.

More specifically, the present invention provides a kit for detecting particular antigens consisting of bacteria of the genus Salmonella, Listeria, E. Coli or Staphylococcus. Said kit is particularly suitable for detecting Salmonella.

In order to further illustrate the present invention and the advantages thereof, the followings examples are given, it being understood that they are intended only as illustrative and in no way limiting.

EXAMPLE 1

1) Preparation of labelled antibody with acridinium derivative and enzyme :

1.1. Preparation of antibody

The antibody used was an anti-Salmonella IgG monoclonal antibody. This antibody was prepared, in a manner known per se, as a pure solution from the culture media after protein A column purification.

1.2. Preparation of acridinium ester

The acridinium derivative used was 4-(2-succinimidyloxy carbonylethyl)phenyl-10-methylacridinium-9-carboxylate methylfluorosulphonate. It was prepared as follows:

Acridine-9-carboxylic acid (1) (5 g) was suspended in redistilled thionyl chloride (15 ml) and boiled under reflux for 3 hours. The solvent was evaporated and dried under reduced pressure to yield orange crystals of acridine-9-carbonyl chloride (2) . Compound (2) (2.3 g) was suspended in anhydrous pyridine (35 ml), benzyl 4-hydroxyphenyl propanoate (9 nmol) added, and the mixture stirred overnight at room temperature.

The mixture was poured into cooled, 1 M hydrochloric acid (250 ml) added and the resulting yellow solid filtered, washed with water and dried under reduced pressure. The 4-(2-benzyloxycarbonylethyl) phenyl-9-acridine carboxylate (3) thus obtained was recrystallised from benzene/cyclohexane to yield yellow crystals (m.p. 135°C).

Benzyl ester (3) (0.46 g) was dissolved in 10 ml of hydrogen bromide/acetic acid mixture (45/55 (w/w)) and the solution stirred for 2 hr at 50-55°C. The solution was poured into 100 ml of water and the resulting yellow solid filtered, washed with water and dried under reduced pressure. The solid was recrystallised from acetonitrile/chloroform (1/1 (v/v)) to yield 4-(2-carboxyethyl) phenyl-9-acridine carboxylate (4) as yellow needles (m.p. 270-273°C).

N-Hydroxysuccinimide was recrystallised from ethyl acetate by the addition of di-isopropyl ether and 62 mg of purified material dissolved in 5 ml of anhydrous dimethylformamide together with 0.2 g of (4) and the mixture cooled to -20°C in CO_2/methanol. N,N-dicyclohexyl carbodiimide (123 mg) was added and the mixture stirred for 2 hour at -20°C and then overnight at room temperature. One drop of glacial acetic acid was then added and the mixture left for a further 30 minutes . The dicyclohexylurea was removed by filtration and the material

obtained by evaporation of the liquor recrystallised from benzene/cyclohexane to yield 4-(2-succinimidyloxycarbonylethyl) phenyl-9-acridine carboxylate (5) as pale yellow crystals.

Compound (5) (234 mg) was dissolved in anhydrous chloroform (15ml) and 0.5 ml of methylfluorosulphonate added. The precipitate which formed after stirring for 18 hours at room temperature was filtered and washed with anhydrous benzene to yield yellow crystals of 4-(2-succinimidyloxcarbonylethyl) phenyl-10-methylacridinium-9-carboxylate methylfluorosulphonate.

1.3. Acridinium labelling reaction :

The reaction can be performed under mild conditions as follows :

1. Dissolve antibody (50 µg of IgG) in 200 µl of labelling buffer (0,2M sodium phosphate, pH = 0,8) ;
2. Add antibody solution to 5 µg of acridinium label, and mix well ;
3. Incubate mixture for 15 minutes at room temperature (25°C) in the dark ;
4. Add 100 µl of quenching buffer (labelling buffer containing 10 mg/ml of lysine monohydrochloride) to mixture, and incubate for a further 5 minutes ;
5. Load mixture onto a 10 ml column of Sephadex^{tm} G25M and collect 15 x 1 ml fractions. A suitable elution and storage buffer is phosphate buffered saline (0,1M, pH 6,3, with 0,15M NaCl) containing 0,05 % (w/v) sodium azide and 0,1 % (w/v) bovine serum albumen.
6. Remove aliquot (1 µl) of each fraction and measure activity in luminometer ;
7. Pool active fractions and store acridinium labelled antibodies at -20°C.

1.4. Enzyme (peroxidase) labelling reaction :

Periodate method. The procedure of Lamoyi & Nisonoff (1983) and Kurstak (1985) was employed. Peroxidase was dissolved in 0,1M $NaHC0_3$ at a concentration of 10 mg/ml ; to this was added 0,05 ml 0,05M sodium periodate solution in distilled water. This was mixed for 2-3 hours in complete darkness. After this period a pasteur pipette was closed by flaming and a glass wool filter was introduced to the constriction. The precipitate of the $(NH_4)_2$ $S0_4$ fraction of the ascites fluid was prepared by adding an equal volume of saturated $(NH_4)_2$ $S0_4$ to ascites fluid, incubating 2 hours at 4°C and then collecting the precipitate by centrifugation at 4.000 x g for 20 minutes. This was dissolved in 0,1M carbonate buffer, pH9,8 at a concentration of 10 mg/ml IgG (determined by A 280). To the activated peroxidase solution was added 1.5 ml of the monoclonal antibody preparation which was added to the sealed pasteur pipette and immediately 0,33 g

dry Sephadex G25M (one-sixth of the combined weight of the solutions) was added. The beads began to swell immediately, concentrating the protein solution approximately two-fold and consuming the excess periodate inside the beads.

This was incubated in complete darkness for 2-3 hours, after which the conjugate (antibody labelled with enzyme) was eluted from the Sephadex (after breaking the tip of the pasteur pipette) by adding 0,1M carbonate buffer, pH9,3. The conjugate was then stabilised by adding 0.5 ml of diethanolamine. The conjugate was stored as aliquots at - 20°C until required.

This method eliminates the problems associated with most periodate methods i.e. the over oxidation of peroxidase which leads progressively to more carboxyl groups which are useless in conjugation, the altering of conformational stability of the enzyme by rendering certain amino acids more hydrophilic and the formation of large polymers also caused by excessive oxidation.

2) Detection of Salmonella by the ELISA procedure :

2.1. Sample preparation and enrichment procedure :

Day 1 : Add 25 g of sample to 225 ml of pre-enrichment broth (eg buffered peptone water). Incubate for 18-24 hours at 37°C. The sample may require blending.

Day 2 : Transfer 1 ml of pre-enrichment broth into 10 ml of selective enrichment broth, normally 10 ml of tetrathionate and 10 ml of selenite cystine are employed. Incubate the tetrathionate broth at 42°C + 0.5 and the selenite cystine broth at 37°C for 18-24 hours.

Day 3 : Transfer 1 ml of tetrathionate broth into 10 ml of modified GN broth and 1 ml of selenite cystine into 10 ml of modified GN broth. Label each modified GN broth accordingly. Incubate for 4-6 hours at 42°C + 0.5. After incubation tranfer 1 ml aliquots of each modified GN broth into 10 ml test tube, mix the combined modified GN broths and autoclave or boil for 20 minutes at 100°C. Cool the broth to room temperature. Mix before performing the ELISA.

2.2. Performing of the ELISA procedure :

The following methodology was pursued :

1. Use the number of wells (in the form of polystyrene wells of a microtiter plate) required to perform the ELISA assay, one well per food sample, one positive control well and one negative control well.

2. Add 100 µl/well of controls and 100 µl/well of autoclaved/boiled samples to the ELISA plate. Incubate the ELISA plates for 30 minutes at room temperature (25°C).

3. Wash each well with a working solution of wash buffer (approximately 250 µl per well) quickly invert the plate emptying contents into a container. Repeat this washing process a further three times with working wash buffer and once with distilled water. Strike the plate facedown several times on a paper towel placed on a flat surface.

4. Add 100 µl/well of the enzyme labelled antibody to each of the sample and control wells. Incubate for 30 minutes at room temperature (25°C).

5. Wash each well as in step 3.

6. Add 100 µl/well of TMB substrate (substrate comprising 3,3',5,5'-tetramethylbenzidine). Incubate in the dark at room temperature for 30 minutes.

7. For automated interpretation : stop the reaction with 100 µl per well of stopping solution. Read within 5 minutes.

8. Measure the plate absorbance using a microplate reader at A 450 nm and record the absorbance values.

3) Detection of Salmonella by the method of the present invention :

3.1. Sample preparation and enrichment procedure :

As previously described under paragraph 2.1. above.

3.2. Performing of the method of the present invention :

The following methodology was pursued :

a) Sample is added (100 µl/well) to the wells of a polystyrene microtiter plate used for immuno assays. This is incubated for 30 minutes at 37°C in the presence of Tris buffer, pH 9,2 ;

b) Microtiter plate is then washed 5 times with PBS buffer containing 0,05% Tween 20 surfactant (polyoxyethylenesorbitan monolaurate) ;

c) Labelled acridinium antibody is added at 100 µl/well and incubated for 30 minutes at 37°C in the presence of Tris buffer ;

d) Plate is washed as in step b) ; and

e) Luminescence measured on LB96P luminometer, which automatically adds an aqueous alkaline solution of hydrogenperoxide to each well of the plate.

4) Comparison of the ELISA method with the chemiluminescent immunoassay (CLIA) according to the present invention for the sensitivity of detection for Salmonella antigens:

Bacterial numbers/ml	ELISA A 450 nm	CLIA RLU
10^6	1.203	1,854,330
10^5	0.241	461,520
10^4	0.128	115,210
10^3	0.108	30,244
10^2	0.108	10,875
Negative	0.108	6,965

A positive result is described as anything significantly greater than the background negative. With the ELISA procedure the negative is 0.108 and a positive result which is significantly higher is taken as 0.3 ; therefore the detection limit is just above 10^5 cells/ml which produce 0.241, i.e. about 2×10^5 cells/ml. With the CLIA procedure the negative result is 6,965 (RLU) and a positive result is considered to be significantly higher than this value. The reading at 10^2 cells/ml is 10,875 which is higher than 6,965 but not significant so the actual detection limit is taken as about 2×10^2 cells/ml. Thus the CLIA method according to the present invention has proved to produce much more than 100 fold improvement on sensitivity when compared to the ELISA.

EXAMPLE 2

This example illustrates a food sample that was artificially contaminated with Salmonella at different concentrations : 10^{-1} containing the highest numbers and then the numbers decreasing by ten fold amounts.

The food were then subjected to an enrichment procedure of about 24 hours or of about 44 hours and then tested using the CLIA method of the present invention which has been previously described under example 3, paragraph 3.2.

The enrichment procedure of about 24 hours was carried out in the following manner :

Day 1 : Add 25 g of sample to 225 ml of pre-enrichment broth (eg buffered peptone water). Incubate for 6 hours at 37°C.

Day 1 : Subculture 10 ml of pre-enrichment broth into 100 ml of GN broth containing 10 µg/ml novobiocin. Incubate for 18 hours at 42.5°C.

Day 2 : Autoclave GN broth and test by CLIA procedure.

The enrichment procedure of about 44 hours was performed according the indications given hereinbefore under paragraph 2.1.

The results were as follows (note: the salmonlla concentrations given below represent serial dilution factors of the salmonella culture):

Salmonella Concentration	CLIA Enrichment : 24 h RLU	CLIA Enrichment : 44 h RLU
10^{-1}	1,985,015	2,132,190
10^{-2}	2,325,400	2,111,235
10^{-3}	2,178,770	2,122,130
10^{-4}	1,863,580	2,204,680
10^{-5}	1,454,290	2,081,650
10^{-6}	1,179,170	1,179,170

From the above table shows, it is clear that the CLIA method of the present invention allows to detect Salmonella bacteria after much shorter time periods for the preparation of samples prior to testing.

While the invention has been described in terms of various preferred embodiments, the skilled artisan will appreciate that various modifications, substitutions, omissions, and changes may be made without departing from the spirit thereof. Accordingly, it is intended that the scope of the present invention be limited solely by the scope of the following claims, including equivalents thereof.

CLAIMS

1. An immunological method for detecting a particular antigen consisting of bacteria present in a mixed culture, in a pure culture or in a contaminated food sample, which comprises the following steps :

a) bringing the sample to be tested into contact with an appropriate solid phase, during a first incubation time sufficient to allow the antigen to be immobilized upon or inside the structure of said solid phase,

b) subjecting the solid phase incubated in the previously described manner to a first wash step to remove the unbound antigen matter,

c) bringing the washed solid phase into contact with a specific antibody, which is labelled by coupling to a chemiluminescent agent comprising an acridinium derivative, the coupling being either in situ or in an extemporaneous manner, said contact being performed during a second incubation time sufficient to allow the labelled antibody to bind to the antigen,

d) subjecting the solid phase (associated in the previously described manner to the complex consisting essentially of the labelled antibody bound to the antigen) to a second wash step to remove any unbound labelled antibody, and

e) placing the solid phase obtained as previously described under point d) above under conditions whereby light may be produced, and the luminescence activity produced may be quantitatively measured with the help of an appropriate device.

2. A method according to claim 1 wherein the particular antigen consists of bacteria of the genus Salmonella, Listeria, E. Coli or Staphylococcus.

3. A method according to claim 1 or 2 wherein the particular antigen consists of bacteria of the genus Salmonella.

4. A method according to claim 1, 2 or 3 wherein the solid phase includes a solid substrate in the form of either a bead, a tube or a microtiter plate well.

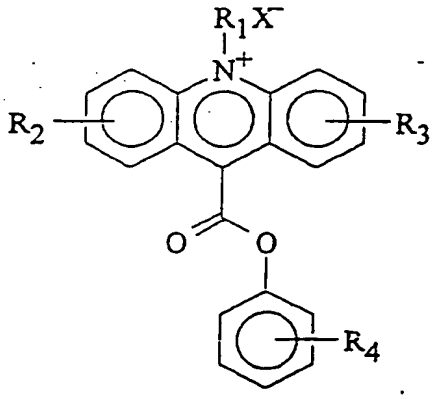
5. A method according to claim 1, 2 or 3 wherein the solid phase includes a solid substrate in the form of either a tube or a microtiter plate well, which is pre-coated with a first unlabelled antibody.

6. A method according to claim 1, 2 or 3 wherein the solid phase consists of a solid substrate in the form of a membrane.

7. A method according to any one of claims 1 to 6 wherein the antibody which is used includes monoclonal antibody belonging to the class of proteins called immunoglobulins (molecules Ig) derived from animal sources.

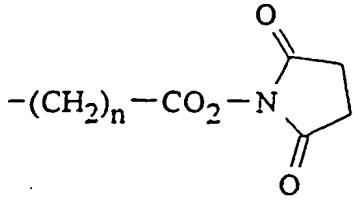
8. A method according to claim 7 wherein the antibody which is used consists of a monoclonal antibody belonging to the class of proteins called immunoglobulins gamma (molecules IgG) derived from rabbits.

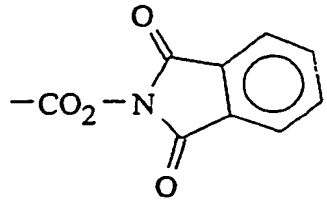
9. A method according to any one of claims 1 to 8 wherein an acridinium derivative used comprises a substituted phenyl ester of an acridinium carboxylic acid having the general formula (I) :



(I)

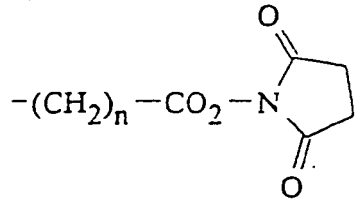
in which : R_1 represents hydrogen, C_1 - C_{10} optionally substituted alkyl, alkenyl or aryl group ; X represents an acceptable salt-forming anion including chloride, bromide, fluoride, sulphite, sulphate, bisulphite, phosphate, orthophosphate, carbonate, bicarbonate, benzoate, sulphonate, methylsulphonate, methylfluorosulphonate ; R_2 and R_3 are hydrogen, amino, substituted amino, carboxyl, alkoxy, nitro or halide substituents ; R_4 is selected from the group consisting of:





-NCS, $-C(=NH_2^+\ X^-)-OR_5$, halide and azide, wherein n represents zero or an integer from 1 to 6 and R_5 represents groups such as R_1.

10. A method according to claim 9 wherein the acridinium derivative used comprises a substituted phenyl ester of 10-methylacridinium-9-carboxylic acid corresponding to formula (I) of claim 9, wherein R_1 is CH_3, R_2 and R_3 are hydrogen, R_4 represents the group :



wherein n represents zero or an integer from 1 to 4, and X represents one of the salt-forming anions as defined in claim 9.

11. A kit for detecting particular antigens consisting of bacteria present in a mixed culture, in a pure culture or in a contaminated food sample, which comprises :

- a solid phase;
- an antibody of the IgG type labelled by coupling with an acridinium derivative; and
- optionally a dilute aqueous solution of an alkaline hydroxide and of hydrogen peroxide.

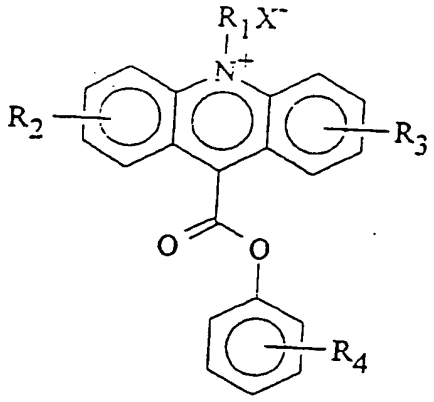
12. A kit according to claim 11 wherein the particular antigens consist of bacteria of the genus Salmonella, Listeria, E. Coli or Staphylococcus.

13. A kit according to claim 11 or 12 wherein the particular antigens comprise bacteria of the genus Salmonella.

14. A kit according to claim 11, 12 or 13 or wherein the solid phase is in the form of either a microtiter plate well or a membrane.

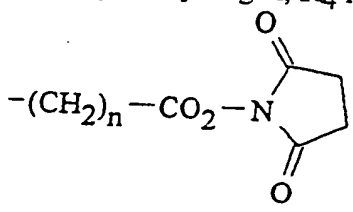
15. A kit according to any one of claims 11 to 14 wherein the antibody is a monoclonal antibody.

16. A kit according to any one of claims 11 to 15 wherein the acridinium derivative comprises a substituted phenyl ester of 10-methylacridinium-9-carboxylic acid corresponding to formula (I):



(I)

wherein R_1 is CH_3, R_2 and R_3 are hydrogen, R_4 represents the group:



wherein n represents zero or an integer from 1 to 4, and X X represents an acceptable salt-forming anion including a chloride, bromide, fluoride, sulphite, sulphate, bisulphite, phosphate, orthophosphate, carbonate, bicarbonate, benzoate, sulphonate, methylsulphonate, methylfluorosulphonate.

INTERNATIONAL SEARCH REPORT

Internati ·l Application No
PCT/EP 94/01586

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N33/569 C12Q1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,87 03690 (MUREX CORPORATION) 18 June 1987 cited in the application see the whole document	1-16
Y	EP,A,0 263 657 (CIBA CORNING DIAGNOSTICS CORP.) 13 April 1988 see the whole document	1-16
Y	WO,A,92 14156 (DYNAGEN, INC.) 20 August 1992 cited in the application see the whole document	1-16
Y	EP,A,0 429 794 (VICAM, L.P.) 5 June 1991 cited in the application see the whole document	1-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 1994	28. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Döpfer, K-P

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INTERNATIONAL SEARCH REPORT

Internati l Application No
PCT/EP 94/01586

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

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